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Inclusion of an alien species in the host range of the Neotropical parasitoid *Hymenoepimecis bicolor* (Brullé, 1846) (Hymenoptera, Ichneumonidae)

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Abstract

In this study we report the first case of an introduced alien host spider species being parasitized and manipulated by an ichneumonid wasp. *Hymenoepimecis bicolor*, previously described parasitizing exclusively *Trichonephila clavipes* (Araneidae), was observed parasitizing the European species *Cyrtophora citricola* (Araneidae) in southeastern Brazil. The cocoon web built by the parasitized spider is composed of a reduced horizontal sheet, which maintains the radial structure. The reduced number of radii and spirals probably reduce the chances of insect interception by these modified structures. In addition, the density of supporting threads is apparently very different between normal and modified webs. The cocoon web spun by *C. citricola* lacks the protective barrier structure usually observed in cocoon webs spun by parasitized females of *T. clavipes*. Our observations are in agreement with several predictions of the ecdysteroid hypothesis and represent an interesting opportunity for further investigation of interactions between these parasitoids and their spider hosts.

Keywords

Host behavioral manipulation, host-parasitoid interactions, introduced species

Introduction

Hymenoepimecis Viereck, 1912 is a genus of parasitoid wasps included in the *Polysphincta* group of genera, members of which are usually referred to as 'polysphinctines' (Hymenoptera, Ichneumonidae, Pimplinae) (Matsumoto 2016). The genus currently comprises 27 species, all of which are restricted to the Neotropical region (Yu et al. 2016; Pádua et al. 2020). Most species were previously reported to parasitize only one host species, but three species have been found to attack two host spiders each: *H. heidyae* Gauld, 1991: *Kapogea cyrtophoroides* (F. O. Pickard-Cambridge, 1904) and *K. sexnotata* (Simon, 1895) (Araneidae); *H. japi* Sobczak et al., 2009: *Leucauge roseosignata* Mello-Leitão, 1943 (Tetragnathidae) and *Mecynogea bigibba* Simon, 1903 (Araneidae); *H. veranii* Loffredo & Penteado-Dias, 2009: *Araneus omnicolor* (Keyserling, 1893) and *A. orgaos* Levi, 1991 (Araneidae) (Gonzaga and Sobczak 2007; Sobczak et al. 2014; Barrantes et al. 2018; Eberhard and Gonzaga 2019). The apparent specificity in the host-parasitoid interactions in the other cases, however, may be a consequence of a lack of information, since parasitoid behaviour has usually been described based on few observations. Conversely, host immobilization and manipulation during the egg laying process of polysphinctines usually involve specific approaching behaviors (see Gonzaga and Sobczak 2007; Takasuka et al. 2009; Kloss et al. 2016) and wasps seems to be very selective on host size (Gonzaga and Sobczak 2007). These factors may reduce the availability of the potential hosts. In addition, most reports of the interactions between *Hymenoepimecis* and their hosts indicate that spiders change their web-building behaviors because of the action of late instar wasp larvae, possibly through the inoculation of the hormone ecdysone or some precursor of this hormone (see Kloss et al. 2017; Eberhard and Gonzaga 2019). These changes result in modified cocoon webs with particular designs, and the survival of pupae of each parasitoid species may depend on specific designs obtained from their hosts. This may be another factor driving host specificity.

To date, all spiders reported as hosts of *Hymenoepimecis* are species native to the geographic range of the parasitoids. In this study we report the first case of a recently introduced host species (*Cyrtophora citricola* (Forsskål, 1775), Araneidae) that was attacked and had its web building behavior altered by a parasitoid previously reared only from a native host spider. We compared the structure of the cocoon web built by the parasitized host with that of normal webs. Additionally, we searched for egg parasitoids of *C. citricola* in two localities in Brazil, namely Uberlândia (MG) and Volta Redonda (RJ), in order to describe possible new interactions between other native parasitoids and this potential new host.

Hymenoepimecis bicolor has a wide geographical distribution, occurring from the Amazon basin (Pádua et al. 2015; Sobczak et al. 2018) to the southern region of Brazil (pers. obs.). This species was previously observed to attack *Trichonephila clavipes* (Linnaeus, 1767) (Araneidae) in Serra do Japi, state of São Paulo (Gonzaga et al. 2010), as well as in several other Brazilian localities, such as Uberlândia (MG), Palmas (TO) and Cascavel (RS) (pers. obs.). The wasp performs direct attacks (Sobczak 2013) after

hovering around the potential host, grasping the spider and introducing the ovipositor into the mouth of the host. The host is then paralyzed, while the wasp searches for and damages eggs previously attached by other individuals, finally depositing her own. The cocoon web spun by the spider shortly before being killed by the parasitoid larva is reduced, lacks spirals, and often presents barrier threads in a position close to that later occupied by the wasp cocoon.

The original distribution of *Cyrtophora citricola* includes Southern Europe, Africa, the Middle East, Pakistan, India, China, and Japan (World Spider Catalog 2022). Levi (1997) reported the first record of individuals collected in South America, in Valle del Cauca, Colombia. The first well-documented record of this species in Brazil was made by Álvares and De Maria (2004), who reported specimens collected in the municipalities of Belo Horizonte (19°52'S, 43°58'W) and Prudente de Morais (19°30'S, 43°07'W), both in the state of Minas Gerais. The specimens examined by these authors were collected in 2000 and 2001. Another possible previous record was made by Alves-Costa and Gonzaga (2001) during a study conducted in August 1998, in an area 80 km north of Manaus (2°30'S, 60°00'W), Amazonas State. These authors identified one of the species included in the study as *Cyrtophora* sp. (possibly *C. citricola*) based on juveniles and the shape of the webs. Later, Álvares and De Maria (2004) mentioned that they searched unsuccessfully for these specimens in the collection of the Instituto Nacional de Pesquisas da Amazônia (INPA) to confirm the identification.

Methods

We found three cocoon webs of *C. citricola* in Volta Redonda, RJ. The first one was attached to the branches of a tree (*Anadenanthera colubrina*, Fabaceae), at a height of approximately 2.5 m from the ground, close to a small river within the urban area (22°31'24.17"S, 44°05'42.62"W). The other two were located within the area of the city zoo (Zoológico Municipal de Volta Redonda) (22°31'56.18"S, 44°06'12.74"W), attached to perennial shrubs (*Euphorbia milii*, Euphorbiaceae), both at heights of about 1 m. The first cocoon web was discovered on August 8, 2019 and the second on July 16, 2021. We located eight other webs of unparasitized individuals of *C. citricola* in a transect of 5 × 200 m from the site where we found the first cocoon web, on the same day. All egg sacs of these spiders were collected. The cocoon web was photographed and the cocoon was carefully removed and maintained in the laboratory until the emergence of the adult wasp. The second cocoon was collected from a damaged web and we kept the cocoon in the laboratory until the emergence of the adult, but no information was collected on the web structure. The third cocoon web had a larva in its last instar attached by its dorsal tubercles on a web thread. We observed the beginning of cocoon construction by the larva and collected the cocoon 24 hours later to rear the adult wasp in the laboratory.

The egg sacs of another 35 spiders were collected in Uberlândia (18°57'11.37"S, 48°17'15.70"W), MG. This additional sampling was conducted two weeks after col-

lecting the first cocoon (in August 2019), searching for parasitized individuals in another population of *C. citricola* (located about 1000 km from the first locality). All the egg sacs (77 from Uberlândia and eight from Volta Redonda) were opened in the laboratory and the egg masses were dissected to locate and identify possible egg parasitoids. We also counted the numbers of eggs inside 36 of those 77 egg sacs from Uberlândia (120.4 ± 79.9 [39 – 366] (mean ± sd [min – max]).

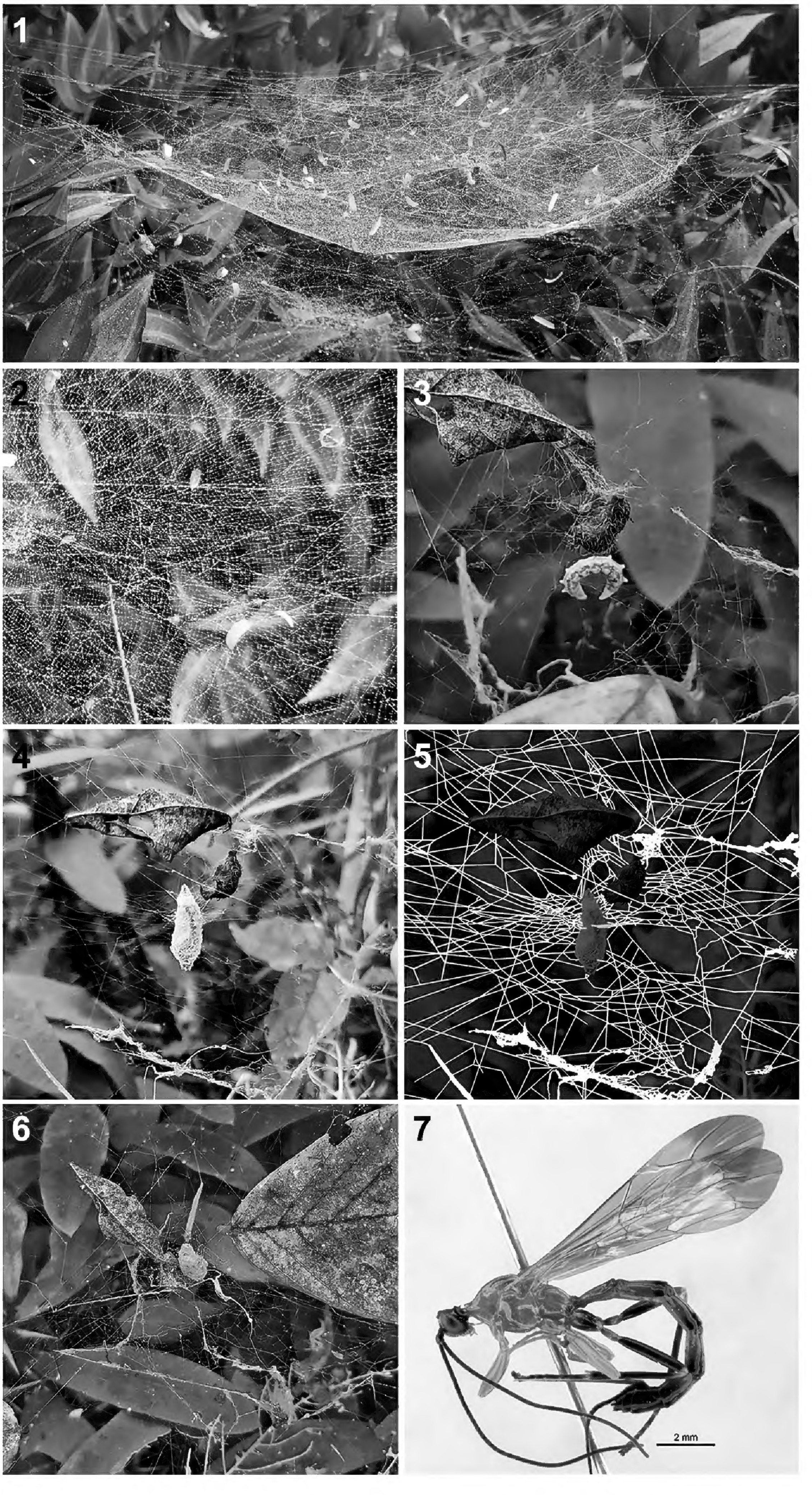
Photographs of normal and cocoon webs spun by *Trichonephila clavipes*, for comparison with webs spun by *C. citricola*, were previously obtained during studies conducted in 2010 in Serra do Japi, Jundiaí, SP, Brazil. The *H. bicolor* images were taken using a Leica DMC4500 digital camera attached to a Leica M205A stereomicroscope and multiple layers were stacked by using the software Helicon Focus 5.3 Pro.

Results

The general structure of the cocoon webs of *C. citricola* differs from those of normal webs, including the reduction of the fine meshed sheet of dry silk, which is always present in webs of unparasitized individuals (Figs 1, 2). The larva consumed the spider at the same location occupied by the host while in its resting position, just below the center of the horizontal radial structure. Its dorsal tubercles then attached to the supporting threads located just above this position (Fig. 3). The cocoon was constructed and remained connected to several radii (Figs 4–6). The *H. bicolor* collected just after cocoon construction emerged as an adult female 15 days later (Fig. 7). Females of *C. citricola* produced 120.4 ± 79.9 eggs (mean ± sd), but we did not find any egg parasitoids or egg predators within these egg masses.

Discussion

The observation reported here is, as far as we know, the first record of a recently introduced alien host being parasitized by a species of *Hymenoepimecis* (Eberhard and Gonzaga 2019). One idiosyncrasy of the interaction between *H. bicolor* and its native host, *T. clavipes*, is that adult and sometimes subadult host females are much larger than the juveniles usually attacked (Sobczak 2013). Consequently, when most spiders within a population reach body sizes above the ideal, there is a pressure for wasps to attack unsuitable individuals (specifically large and potentially dangerous females or small adult males). In fact, Sobczak (2013) observed *T. clavipes* males carrying two larvae of *H. bicolor*. The same pressure may have directed the first attempts of *H. bicolor* to parasitize *C. citricola*, a new potential host within the size range usually selected for oviposition. In addition, individuals of both host species do not construct any form of shelter in their webs, remaining exposed during the day.

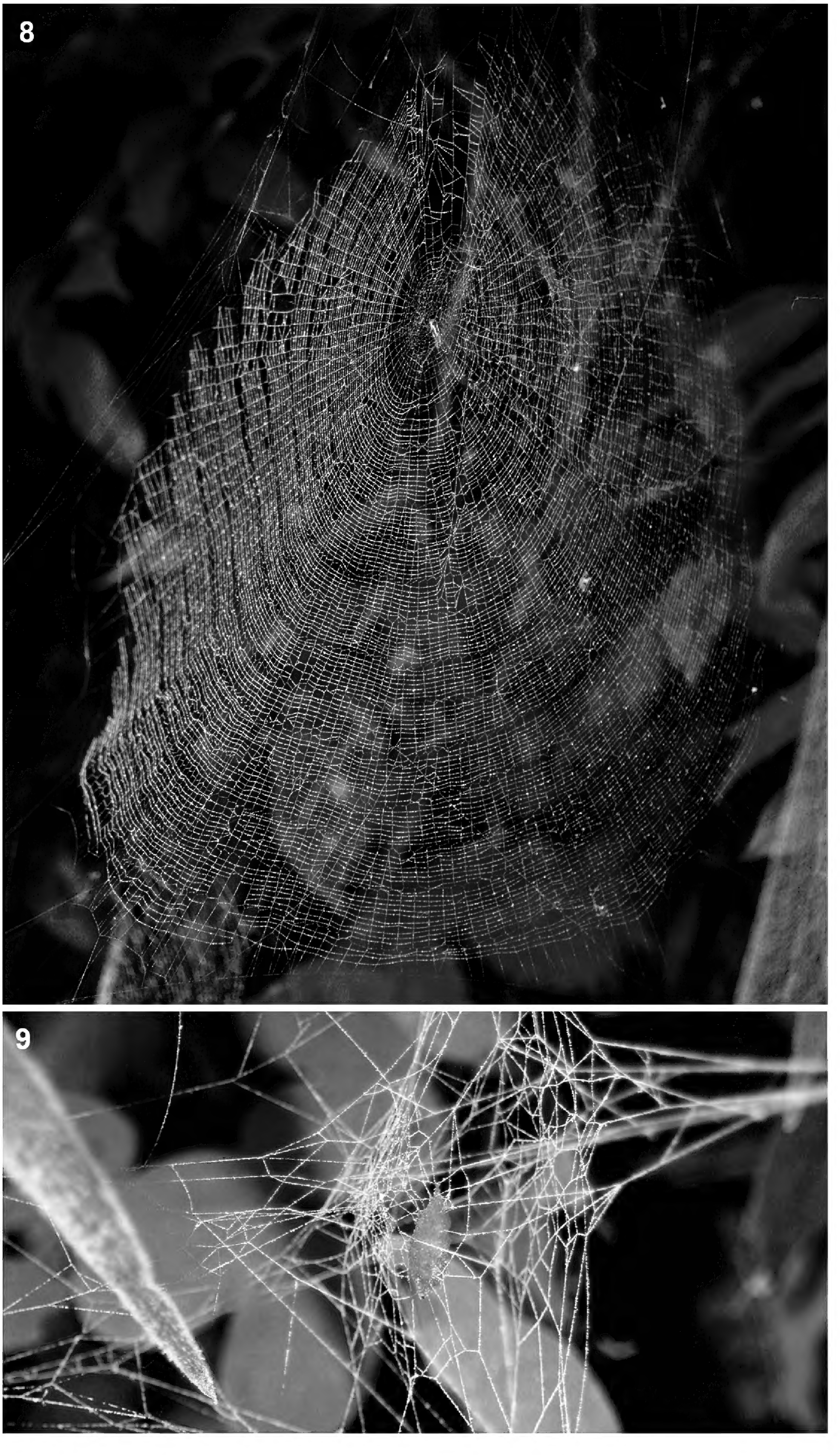


Figures 1–7. 1 normal web structure of *Cyrtophora citricola* **2** detail of the fine meshed sheet of dry silk of a normal web. **3** larva in its last instar, attached to the web by the dorsal hooks **4, 5** frontal view of a cocoon web with a cocoon attached (in **5** the web threads are highlighted) **6** upper view of the same cocoon web **7** female of *Hymenoepimecis bicolor.*

The selection of a new host species is an interesting occurrence because it has implications for our understanding of the plasticity of attacking and subduing behaviors of these wasps and in the mechanism of host behavior manipulation. New interactions between this introduced species and native egg parasitoids or predators, however, were not observed in the studied populations. Currently, records of egg parasitism of *C. citricola* are restricted to Europe (by *Philolema palanichamyi* Narendran, 1994 – Eurytomidae, and *Pediobius pyrgo* (Walker, 1839) – Eulophidae) (Chuang et al. 2019) and to Yemen (by *Eurytoma cyrtophora* Zerova et al., 2008 – Eurytomidae) (Zerova et al. 2008, but see Chuang et al. 2019 on this identification). Further sampling of Brazilian populations will be conducted to investigate the possibility of the establishment of such interactions.

Manipulation of host *T. clavipes* behavior by *H. bicolor* usually results in cocoon webs presenting barrier threads surrounding the position originally occupied by the spider (Gonzaga et al. 2010) (Figs 8, 9). In the two-dimensional normal orb webs of unparasitized individuals, this position is usually exposed (Fig. 8). The increased complexity of this region may be important to reduce cocoon susceptibility to predators and/or hyperparasitoids, and also to increase stability of the web during the wasp's development, as the cocoon is suspended from the web. This new protective component is absent from cocoon webs spun by *C. citricola*. In this case, however, the position occupied by the cocoon is already surrounded by several supporting threads in the normal webs and the connection of the cocoon to the radial threads ensures its stability. As in cocoon webs of *T. clavipes*, the reduction of web components associated with prey interception and retention is the main characteristic of cocoon webs spun by *C. citricola*. This alteration may reduce the risks of web destruction by struggling insects and is often observed in cocoon webs spun by several hosts of polysphinctines (e.g., Eberhard 2000; Gonzaga and Sobczak 2007, 2011; Korenko et al. 2015; Gonzaga et al. 2016).

Eberhard and Gonzaga (2019) listed eight theoretical predictions derived from the ecdysteroid hypothesis (i.e., modifications in web structure induced by parasitoids included in the *Polysphincta* genus group occur as an effect of the injection of ecdysteroids or some precursor of this substance). The case described here corroborates at least four of these predictions: i) strict host species specificity in the wasps should be rare; ii) the phylogenies of the wasps and the spiders that they parasitize should differ; iii) wasps of a single species should induce cocoon web designs that differ widely when they parasitize spiders with different natural histories; and iv) cocoon web design should be adjusted appropriately to the natural history of the spider species to provide protection. A fifth prediction is that cocoon webs should provide increased stability or protection for the cocoon of the wasp. We cannot be sure about this prediction based on a few observations, but the apparently stable structure of *C. citricola* webs likely requires little modification to offer protection to the cocoon (unlike the bidimensional design of *T. clavipes* webs). As previously mentioned, however, the cocoon remains suspended by several threads of the radial structure and probably is subject to a relatively reduced risk of damage or falling due to the action of intercepted insects. Further observation and experimental studies on this new system will clarify the generality of this finding and provide additional relevant information on the extension and limits of manipulation in this particular case.



Figures 8–9. 8 normal web of *Trichonephila clavipes* **9** cocoon of *H. bicolor* attached to a cocoon web spun by *Trichonephila clavipes* (photographs previously obtained in Serra do Japi, Jundiaí, SP).

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